THE OPEN FIELD OF PHARMACOLOGY

S. GARATTINI

Istituto di Ricerche Farmacologiche, Mario Negri, Via Eritrea, 62, Milano, Italy

Contemporary pharmacologists are in the same position as experimental animals in an open field test. Animals are placed on a large table and their interest in the surroundings may be measured by their number of explorative movements. In some modifications of this test the table is full of holes and the "curiosity" of the animals is evaluated by the number of times in which the animals put their heads in the holes. We are now more or less in the same situation. Modern pharmacology is like an open field where we learn new things every moment and where our drugs always become more complicated in their number of sites of action and in their multiple interactions with the living organism. We must therefore admit that our knowledge is still very limited and that we know only an infinitesimal part of what should be known in order to predict therapeutic effects of drugs and to avoid their toxic actions.

Nevertheless the history of drugs has shown that important results may be achieved even if the mechanism of action of new drugs is not fully elucidated. But we must face two different aspects of the same problem at the same time: on one hand we must be careful about possible toxic actions of new or known drugs and on the other we must avoid a too critical approach that would paralyse progress. Even if the therapeutic armory is overflowing with new drugs we must admit that many diseases are still waiting effective remedies. It is enough to mention cancer, atherosclerosis, cardiac infarctions and schizophrenia in order to emphasize how limited are our therapeutical means in combating diseases which represent the major causes of death or disability of mankind. But even diseases which are relatively rare but severe such as multiple sclerosis or certain types of muscular dystrophies are still without therapy and they require a coordinated effort. In this situation the governments and the pharmaceutical industries are justly concerned about the possibility of the appearance of a new "thalidomide". However, instead of stimulating new and deep knowledge the temptation of many official agencies is to complicate the number of assays and bureaucratic procedures before releasing any new drug even for limited clinical evaluations.

Furthermore, the request of prolonged treatments using several animal species and high doses of the drug under study is often a form of defence rather than the result of a precise scientific judgement about the importance of such studies for the extrapolation of data from animals to men. In this way we may create at the experimental level problems which will never exist in the clinic or we may miss important aspects of drug toxicity.

An important point for the discussion of these problems is related to the modern findings of biochemical pharmacology. It may be stated that the
concentration of a drug at the receptor sites—the point where the drug is supposed to exert its therapeutic or toxic effects—is not always directly related to the administered dose. Figure 1 indicates in fact that a drug reaches the receptors only after a number of interactions with membranes and metabolic processes. All these factors are not constant but they change in relation to the species, the strain, the age and the sex. Even when these variables are kept constant, the presence of other drugs, the previous exposure to other unrelated chemicals, the onset of pathological conditions and the intake of different foods may result in changes at the receptor sites or in changes of drug kinetics and metabolism which ultimately determine the concentration of the drug at the receptor sites.

For the moment our ignorance about receptor sites does not permit quantitative considerations. However, it is now possible to investigate some aspects concerning the processes influencing drug kinetics and metabolism. This is possible by the use of advanced physico-chemical techniques such as spectrofluorometry, gas chromatography, radioisotopes and more recently mass spectrometry applied to pharmacology. Many drugs when, after injection reach the circulation, do not remain intact but are transformed into other compounds. In several cases the metabolic pathways are multiple and the number of possible metabolites originating from a single drug may be more than a hundred as in the case of chlorpromazine. Usually these metabolic transformations serve the purpose of increasing the water solubility of drugs which are originally liposoluble and therefore cannot be excreted.

One system responsible for drug metabolism is now relatively well known. It is localized in the liver in the so-called endoplasmic reticulum, a tubular network spreading throughout the cell. When the tubular system is separated from the rest of the cell it breaks and forms vesicles known as microsomes. In the membrane of these microsomes the enzymes called “mixed oxygenases” are located, which metabolize foreign compounds. The term foreign compound is not completely precise since recent evidence suggests that these enzymes may also metabolize endogenous substances such as steroids. These
enzymes, which require a system generating NADPH and several cyto-
chromes, may hydroxylate, dealkylate, or reduce a number of drugs\(^2\). The result of these reactions may lead to compounds which show less activity, more activity or other types of activity in respect to the parent compound. Depending therefore upon the type and the intensity of these enzymes the pharmacological actions of the administered compound may be considerably modified.

Examples of this situation are well known in pharmacology. For instance amphetamine is transformed in rats into \(p\)-hydroxyamphetamine, a compound which shows less stimulant and central effects than the parent compound\(^4\). Similarly, the hydroxylation of pentobarbital results in the loss of its narcotic activity\(^5\). Imipramine is \(N\)-demethylated to form desipramine, a compound provided with strong antidepressant activity\(^6\). Experimentally, it can be shown that the antireserpine activity of imipramine depends upon the level of desipramine in brain. In fact, if \(in\ vivo\) the demethylation is blocked by suitable drugs, such as SKF 525 A, the antireserpine effect of imipramine is considerably decreased (see Table 1).

**Table 1.** Hyperthermic effect induced by imipramine and desipramine in reserpinized rats

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Pretreatment (mg/kg i.os)</th>
<th>Treatment (mg/kg i.p.)</th>
<th>Thermic index (°C) ± S.E.</th>
<th>Brain concen (µg/g) ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Saline</td>
<td>Saline</td>
<td>(-1 \pm 0.4)</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>SKF 525 A 30</td>
<td>Saline</td>
<td>(-1 \pm 0.6)</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>Saline</td>
<td>Imipramine 20</td>
<td>(+7.8 \pm 1.2)</td>
<td>5.1 (\pm 0.9)</td>
</tr>
<tr>
<td>6</td>
<td>SKF 525 A 30</td>
<td>Imipramine 20</td>
<td>(-1 \pm 0.8)</td>
<td>8.5 (\pm 1.2)</td>
</tr>
<tr>
<td>6</td>
<td>Saline</td>
<td>Desipramine 15</td>
<td>(+7.5 \pm 1.4)</td>
<td>*1.9 (\pm 0.4)</td>
</tr>
<tr>
<td>6</td>
<td>SKF 525 A 30</td>
<td>Desipramine 15</td>
<td>(+5.4 \pm 1.4)</td>
<td>*37.3 (\pm 2.2)</td>
</tr>
</tbody>
</table>

Reserpine (5 mg/kg i.v.) was given 16 h before the pretreatment. SKF 525 A was given orally 1 hour before the treatment (saline, imipramine, desipramine).

Thermic index was calculated by adding the change of body temperature induced by saline or imipramine or desipramine after 30, 60, 90 and 120 min.

Desipramine and imipramine were determined in the whole brain 2 hours after their administration.

\(\dagger\) \(p<0.01\) versus saline + imipramine group.

\(*\) \(p<0.01\) versus control groups.

Even more dramatic is the case of parathion, an insecticide which becomes very toxic only when it is transformed into paraoxon\(^7\), a strong inhibitor of cholinesterases. Recently we have been interested in the metabolism of diazepam (I), a tranquilizer, anxiolytic drug. This compound (see Figure 2) is transformed into an \(N\)-demethylated compound (\(N\)-demethyl-diazepam) (II) and into a hydroxylated compound (\(N\)-methyl-oxazepam) (III). These two compounds may be then respectively hydroxylated and \(N\)-demethylated to form a common metabolite known as oxazepam (IV). These three metabolites have been isolated \(in\ vivo\) after administration of diazepam\(^8\) but they can be easily found in more simple systems such as the isolated perfused liver\(^9\) or the fortified liver microsomes\(^10\) (see Figure 3). It is interesting to note that the three metabolites show considerably reduced toxicity in respect to diazepam although they are at least as active as the parent compound in terms of anticonvulsant activity (see Table 2)\(^11\). These few given examples
Figure 2. Metabolic transformation of diazepam (I) into N-demethyldiazepam (II); N-methyloxazepam (III) and oxazepam (IV).

Figure 3. Formation of N-demethyldiazepam (■), N-methyloxazepam (▲), and oxazepam (▲) when different concentrations of rat liver microsomes (105 000 g fraction; 1 ml = 24 mg protein) are incubated for 1 h with diazepam (●).
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demonstrate how important the rate of metabolism may be in determining
the activity and the toxicity of a given drug. It should be now added that
this enzymatic activity responsible for metabolism of drugs is not constant
but changes according to a large number of variables.
First of all, certain enzymatic steps are characteristic of given species and
may not be similar in different species. For instance the \( p \)-hydroxylation of
amphetamine is typical for rats\(^{12} \) but is not present in guinea pigs, mice or
dogs and only very limited in man\(^{13} \).

<table>
<thead>
<tr>
<th>Time between</th>
<th>Maximal dose of metrazol (mg/kg i.p.) inhibited by at least 50%* in mice pretreated with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diazepam</td>
</tr>
<tr>
<td>1 min</td>
<td>298-7</td>
</tr>
<tr>
<td>1 h</td>
<td>207-4</td>
</tr>
<tr>
<td>5 h</td>
<td>172-8</td>
</tr>
<tr>
<td>15 h</td>
<td>120-0</td>
</tr>
</tbody>
</table>

Control animals injected with the solvent were all killed by 100 mg/kg i.p. of metrazol.
*The doses of metrazol were given beginning with 100 mg/kg i.p. with an increase of a factor 1-2. At least 6 mice for each dose of metrazol were used. Drugs were given at the dose of 5 mg/kg i.v. The parameter used for measuring antimetrazol activity was the mortality.

Even in the same species there may be genetic differences according to
the strain employed. This observation applies also to humans. Acetylation
of isoniazid may be fast or slow according to genetic reasons. Subjects which
are slow acetylators tend also to develop more toxic effects, such as poly-
neuritis, than fast acetylators\(^{14} \), probably because the hydrazine moiety
of isoniazid may inactivate pyridoxal dependent enzymes\(^{15} \) which are
important for the function of peripheral nerves. Price Evans has recently
shown that another hydrazine derivative, phenelzine, a monoamineoxidase
inhibitor, induces more side effects in slow than in fast acetylators\(^{16} \).

Sjöqvist and Hammer have recently reported that some subjects develop
side effects after administration of desipramine probably because they lack
hydroxylating enzymes and therefore tend to accumulate high levels of
desipramine in blood\(^{17} \). The same subjects also show high plasma levels of
nortriphyline and a long half life of oxyphenyl butazone\(^{18} \). In an extensive
investigation on diazepam we found that the blood levels of this drug after
a single administration ranged from low to high values and that subjects
with high levels showed always severe drowsiness (see Table 3). Better known
are the severe toxicities of succinylcholine or procaine, which are linked to
the lack of pseudocholinesterase in the plasma of a small percentage of a
population\(^{19} \). Drug metabolism however, does not always explain these
differences. For example, C3H mice are almost insensitive to the action of
amphetamine and yet they show brain levels of amphetamine comparable
to C57Bl/6N or Swiss mice both of which are quite sensitive to the stimulating
effect of this amine (see Table 4).

The microsomal enzymes show a different level of activity also within the
same strain of animals because of the presence of the circadian rhythm.
It has been shown, for instance, by Radzialowski and Bousquet that microsomal enzymes show a different degree of activity for the metabolism of aminopyrine, hexobarbital and p-nitroanisole during the different hours of the day. The half life of metyrapone, a compound used for diagnostic purpose in endocrinology is strikingly different when the chemical agent is given to rats during the day or the night (see Figure 4). This may perhaps explain why the toxicity of this agent follows a daily rhythm as reported by Ertel, Halberg and Ungar. In fact it has been reported that metyrapone shows a different effect in patients if given in the morning or in the afternoon. Furthermore, preliminary experiments show that the half-life of exogenous cortisol in plasma is considerably higher in the morning than in the afternoon.

Up to now we have mostly considered the effect of a drug given in single dose, but changes in activity and in metabolism may occur if the drug is given more than once. It is well known, for example, that a repeated administration of hexobarbital in rats results in a decreased hypnotic effect of this drug due to stimulated metabolic activity of the microsomal enzymes (this effect is called induction). Therefore, the brain levels of hexobarbital decrease with time although doses of administration are equal with time. Similar results have been obtained with amphetamine. The significance of these findings is quite important in studies of chronic toxicity. It may be in fact stated that studying the toxicity of a drug in rats over a six month period, will measure in several cases the toxicity of the metabolites rather than that of the injected compound. If the metabolism of the drug in rats is different from that in humans, the chronic toxicity study in rats will

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Table 4. Effects of amphetamine in different strains of mice

<table>
<thead>
<tr>
<th>Strain of mice</th>
<th>Body temperature (°C ± S.E.)</th>
<th>Brain amphetamine (µg/g ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swiss</td>
<td>41.1 ± 0.5</td>
<td>14.7 ± 1.3</td>
</tr>
<tr>
<td>C57B1/6</td>
<td>38.2 ± 0.2</td>
<td>12.6 ± 0.8</td>
</tr>
<tr>
<td>C3H/J</td>
<td>36.7 ± 0.8</td>
<td>12.6 ± 1.9</td>
</tr>
</tbody>
</table>

All animals were injected with d-amphetamine sulphate (7.5 mg/kg i.p.). Determinations of body temperature and brain amphetamine were performed 30 min after drug administration.
have little meaning for human patients. It may actually mask the presence of toxic effects as shown, for example, by Burns et al. These workers observed that phenylbutazone induced gastric ulcers after a single but not repeated treatment, because of the induction of liver enzymes which decreased the level of plasma phenylbutazone\textsuperscript{27}. Paradoxically, it can be said that phenylbutazone decreases its toxicity by prolonging the treatment. Again, not all the cases of resistance or tolerance to drugs are due to changes of drug metabolism. 3\textit{(Pyridyl)}tetrazole is a compound which decreases the level of plasma free fatty acids (FFA) by blocking lipolysis in the adipose tissue\textsuperscript{28}. This compound, which is not metabolized in rats\textsuperscript{29}, becomes rapidly inactive when the treatment is repeated for several days. Figure 5 shows that 3\textit{(pyridyl)} tetrazole level in blood or adipose tissue is similar in sensitive or resistant rats. Other studies suggest that in this case the resistance may be due to the hypersensitivity to the lipolytic effect of noradrenaline present in resistant animals and reported in Figure 6. This effect has been observed also for other lipolytic blocking agents\textsuperscript{30}. The example given suggests another consideration. We should always be very careful in assuming that similar levels in the blood or in tissue may represent similar concentrations of the drug at the receptor sites. In some cases there is a good proportionality between levels in a tissue and a given pharmacological response. For instance, within certain limits and doses the level of brain amphetamine correlates with the increase of body temperature (Figure 7) and the level of

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure4.png}
\caption{Daily variations of the metabolism of Metyrapone (SU 4885) in rats, expressed by half-life ($t_{1/2}$) in plasma at different times. Metyrapone was injected intravenously at the dose of 50 mg/kg (calculated as a base) and the determinations were performed at 5, 15 and 30 min after drug administration.}
\end{figure}
Figure 5. Levels of 3(pyridyl)tetrazole in plasma and in epididymal adipose tissue (on the left) and effect on plasma FFA (on the right) after administration of the drug at the dose of 30 mg/kg i.v. in normal (●—●) or resistant (○—○) rats. Rats were made resistant to the lipolytic blockade exerted by 3(pyridyl)tetrazole by giving the drug for three consecutive days at the dose of 200 mg/kg oral. The experiment was performed on the 4th day.

brain pentobarbital is proportional to the sleeping time (Figure 8). In other cases, such correlation is not evident and this may be due to the fact that in a tissue a drug may be unevenly distributed in respect to the part of a complex organ such as the brain or in respect to the subcellular structures. For instance, the depletion of brain or heart catecholamines does not parallel the levels of brain or heart reserpine. Actually in this case the low levels of reserpine present at the 6th hour after the administration may be more important that the high levels present at shorter times. The suggestion is that the high levels present in unspecific sites may decline more rapidly than the low levels probably bound to the sites of action. If the dynamics of drug metabolism result in different level of the drug in various organs or parts of the same tissue there are then differences in the type of response which follow similar concentrations of the drug. Table 5
Figure 6. Hypersensitivity to noradrenaline in rats made resistant to the lipolytic blockade exerted by 3(pyrindyl)tetrazole. Rats were treated for 3 days with 200 mg/kg oral of 3(pyrindyl)tetrazole. Twenty-four hours after the last treatment the animals were sacrificed and their epididymal fat pads were incubated in vitro in presence of different concentrations of noradrenaline.

Figure 7. Effect of amphetamine 3.5 mg/kg i.p. (O—O), 7.5 mg/kg i.p. (△—△), 15 mg/kg i.p. (●—●) on body temperature in rats. On the right is reported also the level of brain amphetamine (μg/g) in the same animals.
indicates the interaction between desipramine and noradrenaline in various isolated organs of rats. It is evident that depending on the tissue considered, the same concentration of desipramine may inhibit, potentiate or not alter the action of noradrenaline. This seems to be due to the fact that the uptake of noradrenaline, which is inhibited by desipramine\textsuperscript{52, 63}, may play a different role in the various organs for terminating the action of noradrenaline. These considerations may explain why in the same patient the side effects of desipramine at the level of various target organs may be considered sympathomimetic or sympatholytic\textsuperscript{32}. Up to now we have considered only the interactions between the drug and a living organism in relatively controlled conditions. However, when a drug is given to patients, the situation is much more complex because the pathological conditions may modify the effect of a drug. Examples of such an interaction are available. For instance thyroid function may considerably modify the effect of a drug.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Potentiating dose (g/ml)</th>
<th>Inhibiting dose (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vas deferens</td>
<td>$10^{-7}$</td>
<td>$10^{-5}$</td>
</tr>
<tr>
<td>Rat atria</td>
<td>$5 \times 10^{-7}$</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td>Rat colon</td>
<td>no action</td>
<td>$10^{-7}$</td>
</tr>
<tr>
<td>Rat uterus</td>
<td>no action</td>
<td>$10^{-7}$</td>
</tr>
<tr>
<td>Renal artery</td>
<td>$2 \times 10^{-8}$</td>
<td>$2 \times 10^{-7}$</td>
</tr>
<tr>
<td>Caudal artery</td>
<td>$2 \times 10^{-8}$</td>
<td>$2 \times 10^{-7}$</td>
</tr>
</tbody>
</table>
Amphetamine becomes more toxic in hyperthyroid than in hypothyroid animals without any clear relation between the effects and the levels of brain amphetamine. On the contrary, although the metabolism of amphetamine is impaired in tumor bearing animals, the hyperthermic effect of this amine is considerably reduced. This may be because in these animals the stores of triglycerides are empty and the body cannot mobilize FFA.

Barbiturates may become more toxic in tumor bearing animals because the levels of brain pentobarbital are higher than in normal rats or mice with a consequent remarkable increase of the duration of narcosis. In the case of zoxazolamine, a muscle relaxant agent, it could be proved that the increased duration of paralysis present in tumor bearing animals was correlated to impaired liver microsomal activity and an increased level of brain zoxazolamine (see Table 6). Even changes in behaviour may result in a different toxicity of certain drugs. Amphetamine, for instance, is more toxic when animals are kept in prolonged isolation, a condition which determines the onset of aggressiveness in male mice.

During therapeutic treatment, a drug is usually given in combination with other drugs and here is a source of other interactions. A drug may in fact inhibit or induce the microsomal enzymes of the liver and therefore the second drug given will be respectively more slowly or more rapidly metabolized with different consequences according to the pharmacological activity of the metabolites. Several examples of this kind are known. For instance, in man the half-life of diphenylhydantoin is considerably increased if this drug is given in combination with phenyramidol. Phenobarbital on the other hand accelerates the metabolism of several drugs. For this reason the half-life of hydrocortisone in plasma is decreased by about 50 per cent in patients who received an evening administration of phenobarbital for 3 weeks. Much more complex and unpredictable drug interactions may occur if the variability in the metabolism of the single drug is taken into consideration. For instance, it was mentioned before that imipramine administration results in a different level of plasma desipramine.

Table 6. Pharmacological effects and metabolism of zoxazolamine in tumor bearing rats

<table>
<thead>
<tr>
<th>Duration of paralysis (min ± S.E.)*</th>
<th>Controls</th>
<th>Walker*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoxazolamine (μg/g ± S.E.)† in</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>161 ± 5</td>
<td>328 ± 14†</td>
</tr>
<tr>
<td>Liver</td>
<td>3-8 ± 0-3</td>
<td>12-5 ± 0-8†</td>
</tr>
<tr>
<td>Plasma</td>
<td>7-3 ± 1-0</td>
<td>13-7 ± 1-8†</td>
</tr>
<tr>
<td>Zoxazolamine metabolism in vitro‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μg/mg liver protein/h ± S.E.)</td>
<td>3-3 ± 0-7</td>
<td>6-5 ± 1-0†</td>
</tr>
</tbody>
</table>

* Rats were transplanted with Walker carcinosarcoma 16 days before the experiment.
† Zoxazolamine was injected intravenously at the dose of 50 mg/kg. Determinations in plasma and tissues were carried out 6 h after administration.
‡ Zoxazolamine was added in vitro at the concentration of 500 μg, to 9000 g fraction of rat liver and incubated for 1 h.
¶ p < 0.01 with respect to controls.
according to the patient. Recently Smith reported that hydroxylation of amphetamine in different subjects may range from 0.1 to almost 10 per cent of the administered amphetamine. Since amphetamine hydroxylation is blocked by desipramine, it is evident that in subjects with high capacity to hydroxylate amphetamine, the combination of desipramine-amphetamine may be more toxic than in subjects which form only traces of \( p \)-hydroxyamphetamine. These types of interaction may occur more frequently than is usually believed and may perhaps explain some of the toxic effects that, because of our ignorance, are called idiosyncrasies, hypersensitivities or allergic reactions.

Finally, not only drugs but also other chemicals interacting with the organism may affect metabolism and activity of given drugs. For instance, rats pretreated with nicotine metabolize meprobamate more rapidly than controls, probably because nicotine is an inducer of liver microsomes. Caffeine and alcohol may show the same effect in respect to other drugs. And on the contrary, a drug may not be toxic by itself, but because it impairs the metabolism of nicotine, caffeine or alcohol or because through various mechanisms it may increase the pharmacological effects of these compounds it may become a toxic agent. Very well known in this respect is the interaction between disulfiram (Antabuse) and ethanol. In certain subjects chlorpromazine impairs the metabolism of alcohol thus resulting in blood levels of ethanol to be over 50 mg per cent, a level which results in central toxicity. Interactions of administered drugs with food additives, such as certain insecticides which accumulate in adipose tissue and are known to be inducers may be foreseen to increase in the future. A dramatic example of drug interaction with food constituents is represented by the cerebral hypertension observed in subjects treated with monoamineoxidase inhibitors. Foods such as cheese and red wine are rich in tyramine, a sympathomimetic amine which becomes toxic if it is not inactivated by monoamineoxidase. This effect is not observed with imipramine, another antidepressant drug. Actually in this case, the tyramine effect is considerably decreased because imipramine impairs the uptake of tyramine and therefore permits a more rapid inactivation. However, for other sympathomimetics, the picture is different.

Noradrenaline, for instance, shows its normal hypertensive effect in animals treated with monoamineoxidase inhibitors but it is considerably potentiated by a pretreatment with tricyclic antidepressant agents. The variety of factors and conditions able to change drug metabolism and activity makes the definition of what is the toxicity of a drug extremely difficult. In fact, the previous examples should have shown that the same drug at the same dose even in the same subject may considerably change its activity and therefore increase the probability that toxic effects will appear. A drug therefore always presents a potential danger and only an extensive analysis will allow us to assess whether the favourable effects outweigh the undesired actions of a drug. In this respect it should be emphasized again that, besides certain minimal requirements, it may be very dangerous for the public health and the progress of knowledge to rely on stereotyped procedures for the evaluation of potential toxic effects of new drugs.
In this sense the pharmacology is an open field where our curiosity and our investigation may permit the increase of the benefits over the dangers of new drugs for the treatment of old and still uncured diseases.

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